

IAA-OXIDASE ACTIVITY, DOPA-OXIDATIONS AND
INACTIVATION OF DOPA AS IAA-OXIDASIC INHIBITOR
IN *LENS* ROOTS EXTRACTS (*)

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SUMMARY

Four concentrations of *Lens* roots extracts have been studied as to their oxidative capacity on IAA and DOPA. The kinetics of oxidations are quite different according to the concentrations of the extracts. IAA destruction increases with enzymic extract concentration. DOPA oxidation does not run parallelly. Inhibitors of the DOPA reaction are proved to be present in extracts by the inhibition of a purified tyrosinase when *Lens* extracts are added.

Peroxidases of *Lens* are able to oxidize DOPA since H₂O₂ greatly increases the reaction.

DOPA, according to its o-diphenolic structure, acts as an IAA-oxidasic inhibitor.

Preincubation of DOPA with enzymic extract or tyrosinase results in a diminution of the IAA-oxidasic inhibition. H₂O₂ activates the inhibitor neutralisation by the enzymic extract.

The inactivation of DOPA as IAA-oxidasic inhibitor seems thus simply involve oxidation by a polyphenoloxidase or by a peroxidase.

INTRODUCTION

In a previous article [1], the effect of various phenolic compounds on the β -indoleacetic acid-oxidase (IAAO) and the literature on this subject have been presented. It appears that monophenolic compounds are auxin-oxidasic activators while o-diphenolic derivatives act as inhibitors.

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The 3,4-dihydroxyphenylalanine (DOPA) is one of these inhibitors of the IAAO. The mechanism of the inhibitory effect of polyphenols has specially been investigated by RAY [17], KENTEN [10], MACLACHLAN et WAYGOOD [11, 12]. SACHER [18, 19] has shown that the inactivation of a natural inhibitor from *Phaseolus* during the oxidation of IAA involves the enzyme IAAO.

Our present experiments have been conducted to investigate the capacity of *Lens* extracts to oxidize DOPA, alone or with aid of H₂O₂, and the role of tyrosinase and peroxidase in inactivating this inhibitor for the IAAO activity.

MATERIAL AND METHODS

— Preparation of enzymic extracts from *Lens culinaris* Med. roots has been described elsewhere [16]. Tips (12 mm) of primary roots (18 mm) of *Lens* germinated for 48 hours on watered filter paper at 25° C (dark) are freeze-dried and then ground with phosphate buffer pH 6.1. 100 roots are used for 10 ml buffer solution. The mixture is centrifuged (8,000 rpm ; 15 min.).

The supernatant is used as enzymic extract.

— Variations in the constituents of the reaction mixtures (10 ml) are referred to in the paper.

The enzymic incubations are carried out at 30° C (dark) without 2,4-dichlorophenol [8] and MnCl₂ contrary to GALSTON and DALBERG [4] and SACHER [18].

— Colorimetric determination of IAA destruction [16] uses SALKOWSKI's reagent after PILET [13] : 1 ml of the reaction mixture is pipetted into 6 ml SALKOWSKI's reagent and stirred. Optical densities are determined after 20 minutes using a BECKMAN DU spectrophotometer at 535 m μ . Results are expressed in μ g IAA destroyed in the reaction mixtures.

— DOPA-oxidase activities are also determined spectrophotometrically by measuring the increase of absorbancy at 480 m μ . The blanks are the same reaction mixtures where DOPA is replaced by H₂O.

RESULTS

1. IAAO activity has been determined in reaction mixtures containing 0.5, 2, 4 and 6 ml of enzyme solution and 350 μ g IAA

(fig. 1). The kinetics of the IAAO are different according to enzyme concentrations. A lag period before IAA destruction is observed in presence of 0.5 ml plant extract. No lag occurs with 2, 4 or 6 ml enzymic extract. The relationship between IAA destruction by *Lens* roots and enzyme concentration has already been discussed [14, 15, 5].

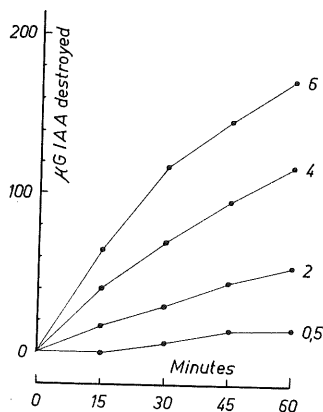


Fig. 1. — Effect of enzymic extract concentration on IAA destruction. Reaction mixtures contain $2 \cdot 10^{-4}$ M IAA ($350 \mu g$), 0.5, 2, 4 or 6 ml enzyme and phosphate buffer pH 6.1 up to 10 ml.

2. DOPA-oxidation has been investigated in reaction mixtures containing 0.5, 2, 4 and 6 ml of the same enzymic extract of *Lens* in presence of DOPA 10^{-3} M. It must be mentioned that the

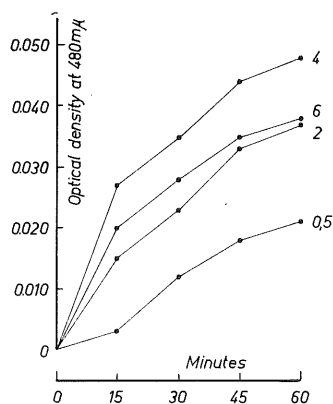


Fig. 2. — Effect of enzymic extract concentration on DOPA-oxidation. Reaction mixtures contain $1 \cdot 10^{-3}$ M DOPA, 0.5, 2, 4 or 6 ml enzyme and phosphate buffer pH 6.1 up to 10 ml.

DOPA-reaction observed is slight, just discernible for the eye. But it exists ; it is greater when the quantity of enzymic extract grows in the reaction mixture to 4 ml. Much enzyme solution (6 ml) gives a slighter reaction (fig. 2).

3. We thought *Lens* roots contain inhibitors of the DOPA-oxidation : the action of the same quantities of extract has been studied on the oxidation of DOPA by purified mushroom tyrosinase (from Sigma Chemical Company).

It can be seen in fig. 3 that addition of *Lens* extract to tyrosinase in the reaction mixtures diminishes DOPA reaction, which indicates the presence of polyphenoloxidasic inhibitors.

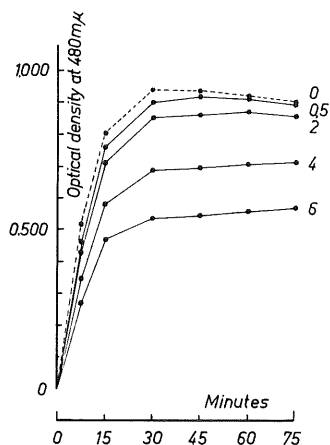


Fig. 3. — Effect of addition of various *Lens* extract concentrations on DOPA-oxidation by purified tyrosinase. Reaction mixtures contain 1.10^{-3} M DOPA, 0,03 mg tyrosinase, 0, 0.5, 2, 4 or 6 ml *Lens* extract and phosphate buffer pH 6.1 up to 10 ml.

4. DOPA peroxidation has also been measured in mixtures containing 0.5, 2, 4 or 6 ml extract and 1 ml H_2O_2 0.2 vol. (fig. 4). It appears that peroxidases of *Lens* [15] are able to oxidize DOPA ; approximatively (in 15 minutes) the reaction is 30 times greater than in absence of H_2O_2 . In these cases, brown colorations of DOPA reaction-products are appreciable and directly related to enzyme concentrations.

It must be mentionned the kinetics of DOPA oxidations with and without H_2O_2 are quite different.

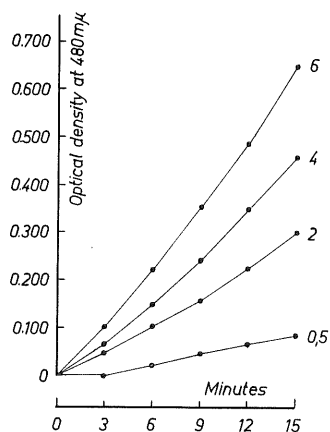


Fig. 4. — Effect of enzymic extract concentration on DOPA-peroxidation. Reaction mixtures contain $1 \cdot 10^{-3}$ M DOPA, 0.2 vol. H_2O_2 , 0.5, 2, 4 or 6 ml enzyme and phosphate buffer pH 6.1 up to 10 ml.

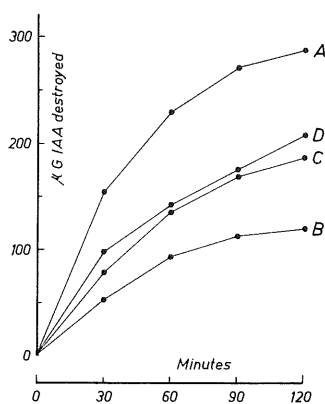


Fig. 5. — Effect of DOPA and of its preincubation with the enzymic extract or with tyrosinase on IAA destruction.

Reaction mixtures are the following :

A : 5 ml enzymic extract + 1 ml H_2O
 + 2 ml buffer + 2 ml IAA 10^{-3} M

B : 5 ml enzymic extract + 1 ml DOPA $5 \cdot 10^{-5}$ M
 + 2 ml buffer + 2 ml IAA 10^{-3} M

C : 5 ml enzymic extract + 1 ml DOPA $5 \cdot 10^{-5}$ M
 + 2 ml buffer + 2 ml IAA 10^{-3} M

D : 5 ml enzymic extract + 1 ml DOPA $5 \cdot 10^{-5}$ M
 + 2 ml tyrosinase (0.5 mg/100 ml) + 2 ml IAA 10^{-3} M.

Components underlined are added at zero time. Others have been for 30 minutes preincubated at $30^\circ C$.

5. The capacity of *Lens* extracts to oxidize DOPA has been regarded as an inactivating mean for this IAA-oxidasic inhibitor. DOPA has been preincubated (30 minutes) with enzymic extract or with tyrosinase before beginning the IAA-oxidasic reaction. Fig. 5 indicates that inhibition produced by DOPA first preincubated with enzymic extract or tyrosinase is very reduced comparatively to inhibition by DOPA added at zero time. Polyphenol-oxidase, if present near IAAO in extract, can thus be regarded as responsible for inactivation of polyphenolic inhibitors of the IAAO.

6. Comparatively in extract alone (fig. 6 I) and in presence of H_2O_2 (fig. 6 II), DOPA inhibition has been studied when added at zero time or after preincubation. Preincubation of DOPA with enzymic extract and H_2O_2 for 30 minutes oxidizes the DOPA (the reaction mixture is coloured) in such a way that it is practically no more inhibitor for the IAAO. (The reduced IAAO activity of *Lens* extract in the presence of H_2O_2 , will be discussed elsewhere).

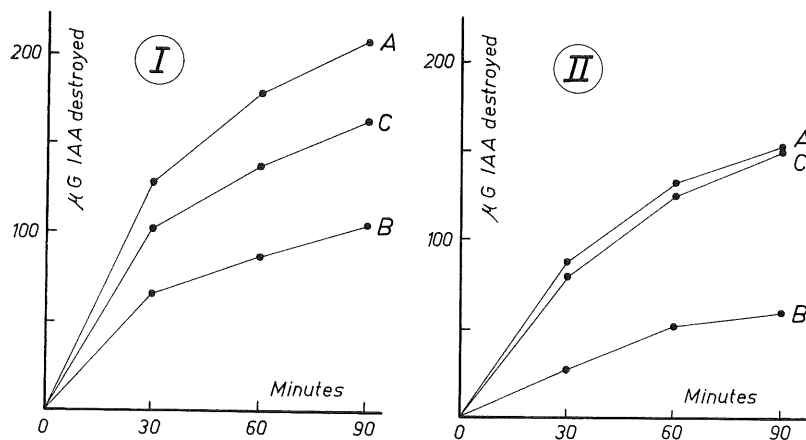


Fig. 6. — Effect of DOPA and of its preincubation with the enzymic extract, alone (I) or in presence of H_2O_2 (II) on IAA destruction.

Reaction mixtures are the following :

A : 5 ml enzymic extract + 1 ml buffer + 1 ml H_2O (I) or 1 ml H_2O_2 0.02 vol. (II) + 1 ml H_2O + 2 ml IAA 10^{-3} M.

B : 5 ml enzymic extract + 1 ml buffer + 1 ml H_2O (I) or 1 ml H_2O_2 0.02 vol. (II) + 1 ml DOPA $5 \cdot 10^{-5}$ M + 2 ml IAA 10^{-3} M

C : 5 ml enzymic extract + 1 ml buffer + 1 ml H_2O (I) or 1 ml H_2O_2 0.02 vol. (II) + 1 ml DOPA $5 \cdot 10^{-5}$ M + 2 ml IAA 10^{-3} M

Components underlined are added at zero time. Others have been for 30 minutes preincubated at $30^\circ C$.

Peroxidase too can be responsible in *Lens* extract for the inactivation of certain phenolic inhibitors of the IAAO.

DISCUSSION

Preincubation of DOPA with enzymic extract of *Lens* diminishes its further IAA-oxidasic inhibitor power. H_2O_2 increases considerably this inhibitor inactivation. With FURUYA and GALSTON [2] and SACHER [18], we can thus conclude to the participation of peroxidase in the inactivation of IAAO-inhibitors.

If IAAO can be considered as a peroxidasic system as first suggested by GOLDACRE [9], GALSTON and coll. [3] and confirmed in many other laboratories [6], the same IAAO would be able to oxidize and neutralise its own phenolic inhibitors.

Inactivation of phenolic inhibitors of the IAAO is not an exclusive property of peroxidase. Tyrosinase also can reduce the IAA-oxidasic inhibition conferred by DOPA by oxidation of the phenol.

The inactivation of IAA-oxidasic inhibitors can thus take place out of the free-radical sequence of the IAA oxidation as proposed by SACHER [18].

Beside the IAA-oxidasic property of tyrosinase in particular conditions [1], we will note here its possible and indirect participation in the IAA destruction by oxidation and so inactivation of the IAAO-inhibitors.

In *Lens* extract, peroxidase must be regarded as the inhibitor inactivating agent. DOPA-reactions in *Lens* extracts without H_2O_2 are slight and tyrosinasic inhibitors are present. *Lens'* peroxidases and a natural peroxide must be questioned as responsible for DOPA-reactions without exogenous H_2O_2 .

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